



Review Article

Cheese as a Functional Matrix for Probiotics: From Strain Selection to Clinical Benefit

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Abstract

Probiotics are defined as live microorganisms that confer health benefits to the host when consumed in adequate quantities. Since their introduction by Élie Metchnikoff in the early twentieth century, these microorganisms have attracted substantial scientific and commercial interest due to their established roles in supporting gastrointestinal health and improving the functional attributes of foods. Within the dairy sector, cheese has emerged as a particularly advantageous carrier for probiotic delivery owing to its high buffering capacity, compact structure and nutrient-rich composition, all of which contribute to enhanced microbial survival during manufacturing, storage and gastrointestinal transit. This review provides an overview of the historical evolution of the probiotic concept and examines the principal bacterial genera employed in probiotic cheeses, including *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus*. The microbiological traits, metabolic activities and adaptive strategies of these microorganisms are evaluated with respect to their influence on cheese composition, biochemical transformations, flavor development and overall sensory attributes. Furthermore, the review synthesizes evidence on the health-promoting effects associated with probiotic cheese consumption, encompassing antimicrobial properties, immunomodulatory activity, cholesterol-lowering potential, anticancer effects and improved lactose metabolism, alongside relevant safety considerations. In summary, probiotic cheese represents an integration of traditional dairy processing with modern functional food innovation. The incorporation of viable probiotic cultures into cheese not only enhances its nutritional and therapeutic value but also provides an effective and palatable vehicle for delivering beneficial microbes within the human diet.

Key words: *Lactobacillus*, *Bifidobacterium*, *Streptococcus thermophilus*, functional dairy products, gut health, microbial viability, cheese ripening, sensory quality, health benefits, lactic acid bacteria

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INTRODUCTION

Probiotics have gained considerable scientific and commercial interest due to their demonstrated capacity to promote human health and enhance the functional and nutritional attributes of foods. The conceptual origin of probiotics can be traced to the early twentieth century, when Metchnikoff¹ proposed that the consumption of fermented milk containing lactic acid bacteria could support health and longevity. Concurrently, Tissier² isolated *Bifidobacterium* from the feces of breast-fed infants and advocated its therapeutic use for intestinal disorders. These seminal findings established the groundwork for the contemporary understanding of probiotics. The term "probiotic," meaning "for life," was introduced by Werner Kollath in 1953 to describe essential substances required for healthy development, in contrast to antibiotics³. Subsequently, Lilly and Stillwell⁴ defined probiotics as microbial products that stimulate the growth of other microorganisms, while Parker⁵ highlighted their role in maintaining intestinal microbial balance. Fuller⁶ later refined the definition to "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance." The current FAO/WHO definition describes probiotics as live microorganisms which, when administered in adequate amounts, confer health benefits to the host⁷.

Among the diverse microorganisms identified to date, *Lactobacillus* and *Bifidobacterium* remain the most extensively studied genera, largely due to their long-standing history of safe use and robust physiological adaptation to the gastrointestinal environment⁸. *Bifidobacteria* are Gram-positive, non-motile, anaerobic rods that ferment carbohydrates via the fructose-6-phosphate shunt, yielding lactic and acetic acids in a 2:3 ratio^{9,10}. *Lactobacilli* are acid-tolerant organisms commonly isolated from plant materials, fermented foods and mucosal surfaces of animals¹¹. Both genera are frequently incorporated into fermented dairy products owing to their ability to produce organic acids and antimicrobial compounds that enhance

food safety and extend shelf life. Cheese represents an optimal matrix for probiotic delivery because its buffering capacity, high fat content and dense microstructure protect microorganisms during storage and gastrointestinal passage^{12,13}. Several cheese varieties, including Cheddar, Gouda, Ras and Minas, can sustain viable probiotic populations above 10 CFU g⁻¹ throughout ripening^{14,15}.

Beyond survival, probiotic cultures may also modulate cheese biochemistry by influencing proteolysis and lipolysis, thereby affecting flavor and texture development^{16,17}. In addition to technological improvements, probiotic cheeses are associated with a range of potential health benefits, including enhanced lactose digestion, reduced serum cholesterol, inhibition of pathogenic microorganisms and modulation of immune responses¹⁸⁻²¹. Clinical studies further report positive effects on oral health, such as decreased *Streptococcus mutans* and yeast counts in saliva^{22,23}. Collectively, the incorporation of probiotics into cheese integrates traditional dairy technology with modern functional food innovation. A comprehensive understanding of the microbiological, technological and health-related dimensions of probiotic cheese is essential for developing high-quality dairy products that align with consumer expectations for nutrition, safety and overall well-being.

Historical overview: The concept of probiotics can be traced to the early twentieth century, reflecting a progressive shift from empirical observations to a scientifically structured discipline (Table 1). The initial foundation was established by Élie Metchnikoff in 1907, who proposed that the regular consumption of fermented milk containing lactic acid bacteria could promote health and longevity—a hypothesis inspired by the exceptional lifespan of Bulgarian peasants who routinely consumed such foods¹. Earlier, in 1900, Henri Tissier² had isolated *Bifidobacterium* from the feces of breastfed infants and recommended its use in preventing infantile diarrhea. The term "probiotic," meaning "for life," was later introduced by Werner Kollath in 1953 to describe essential organic substances required for healthy development, in contrast to antibiotics³.

Table 1: Historical milestones in the evolution of the probiotic concept

Year	Milestone	References
1907	First work proposed the health benefits of consuming fermented milk containing the probiotic.	Metchnikoff ¹
1953	First work that used the term probiotic, meaning "for life," refers to organic and essential substances necessary for healthy development, contrasting it with antibiotics	Hamilton-Miller ³
1965	First work defined probiotics as "a substance secreted by one microorganism that stimulates the growth of another"	Lilly and Stillwell ⁴
1974	First work defined probiotics as "organisms and substances which contribute to intestinal microbial balance"	Parker ⁵
1989	First work referred to probiotics as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance"	Fuller ⁶

Subsequent definitions contributed to the refinement of the probiotic concept. Lilly and Stillwell characterized probiotics as “a substance secreted by one microorganism that stimulates the growth of another,” highlighting intermicrobial interactions rather than solely nutritional effects⁴. Parker later expanded this perspective by defining probiotics as “organisms and substances which contribute to intestinal microbial balance,” thereby emphasizing the role of viable microorganisms in maintaining gut homeostasis⁵. A major conceptual advancement was proposed by Fuller, who defined probiotics as “live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance,” integrating microbial viability, host interactions and measurable health outcomes⁶.

Significant developments occurred during the 1990s, when advances in molecular biology and microbial ecology enabled the identification of specific probiotic strains—particularly from *Lactobacillus* and *Bifidobacterium*—with well-documented health benefits^{18,24}. This period also marked the expansion of probiotics from traditional fermented foods such as yogurt and kefir to encapsulated dietary supplements formulated to enhance stability and survival during gastrointestinal transit²⁵. A major milestone was reached in 2001 when the FAO/WHO Expert Consultation established the widely accepted definition of probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”⁷.

Since the adoption of this definition, extensive research has validated the beneficial effects of probiotics on gut microbiota composition, immune function and the prevention or mitigation of various diseases. Today, most commercial probiotic formulations rely on *Lactobacillus* and *Bifidobacterium* species, which are generally recognized as safe (GRAS) due to their long history of safe consumption and substantiated health-promoting properties¹³.

Key probiotic microorganisms

Bifidobacteria: Bifidobacteria constitute one of the most important groups of probiotic microorganisms, widely recognized for their essential contribution to intestinal health. They were first isolated and described by Tissier² at the Pasteur Institute, who identified them as dominant bacteria in the feces of breastfed infants and initially designated the type species as *Bacillus bifidus*¹⁰. Over time, the taxonomy of the group evolved, with several nomenclatures—including *bifidus*, *bifidum*, *bifida* and *parabifidus*—used to describe members of this genus. The term *bifidus*, derived from the Latin word meaning “split” or “divided,” reflects the characteristic branching morphology exhibited by these bacteria under nutrient-limited conditions²⁶.

Morphologically, *Bifidobacterium* species are pleomorphic, Gram-positive, non-motile and non-spore-forming rods that may appear slender, slightly curved, or branched. They frequently form star-like clusters, Y-shaped structures, or palisade arrangements, with morphological variation influenced by the growth medium²⁷. On solid media, they typically produce smooth, convex, glistening colonies with entire margins and a white to cream coloration. Although considered obligate anaerobes, some species exhibit partial aerotolerance; for example, *B. longum*, *B. breve* and *B. infantis* can withstand limited oxygen exposure by slowing their metabolic activity while sustaining acid production²⁸.

Optimal growth conditions vary with host origin. Human-associated strains generally thrive at 36–38°C, whereas animal-derived species grow better at slightly elevated temperatures (41–43°C). Pre-incubation at 37°C for approximately 18 hrs has been reported to improve their viability during refrigerated storage²⁹. Their preferred pH for growth is between 6.5 and 7.0, with substantial inhibition occurring at both acidic (pH 4.0) and alkaline (pH 8.0–8.5) extremes, depending on the species³⁰.

Metabolically, *Bifidobacterium* species ferment a wide variety of carbohydrates through the distinctive fructose-6-phosphate shunt pathway, producing lactic acid as the principal end product without generating carbon dioxide (CO₂). They typically utilize ammonia (NH₃) as a nitrogen source. The organic acids produced during fermentation contribute to intestinal health by suppressing pathogenic microorganisms and enhancing gut motility. However, in cultured dairy products, their growth can be inhibited by lactic acid and other metabolites produced by lactic acid bacteria³¹. To date, approximately 30 *Bifidobacterium* species have been isolated from diverse sources, including humans, animals, insects, soil and fermented foods. Among these, six human-origin species—*B. bifidum*, *B. lactis*, *B. adolescentis*, *B. breve*, *B. infantis* and *B. longum*—are most frequently incorporated into fermented dairy formulations. Collectively, bifidobacteria represent key probiotic organisms characterized by their anaerobic metabolism, acid production and broad health-promoting effects within the human gastrointestinal tract.

Lactobacilli: The genus *Lactobacillus* encompasses a diverse group of probiotic bacteria commonly found in carbohydrate-rich environments, including plant materials, fermented foods and the mucosal surfaces of humans and animals—particularly within the oral cavity and intestinal tract³¹. These microorganisms are strictly fermentative, ranging from aerotolerant to anaerobic and are well adapted to acidic environments, demonstrating both acidophilic and aciduric characteristics.

Nutritional and metabolic characteristics: *Lactobacillus* species possess complex nutritional requirements due to their inability to synthesize porphyrins, which are essential for heme-dependent enzymatic activities. Nevertheless, certain strains can utilize porphyrins from the environment, enabling limited catalase activity as well as nitrate-reducing or cytochrome-associated reactions³².

Morphological characteristics: *Lactobacillus delbrueckii* subsp. *bulgaricus*, a representative species and an important starter culture in yogurt fermentation, is a Gram-positive, non-motile, non-spore-forming rod with rounded ends. Cells occur singly or in short chains of three to four cells, although longer chains may develop during the stationary phase, forming V-shaped or palisade arrangements. Older cells may contain metachromatic granules that are more prominent when stained with Gram or methylene blue dyes³³.

Growth and fermentation behavior: *L. delbrueckii* subsp. *bulgaricus* is a homofermentative organism that produces predominantly D(-)-lactic acid as the major end product of carbohydrate metabolism. It ferments sugars such as glucose, fructose and lactose but does not utilize arginine. The bacterium is catalase-negative, facultatively anaerobic and highly sensitive to oxygen. Optimal growth occurs near 45°C and the organism tolerates temperatures up to 50-55°C. During fermentation, culture pH typically declines to 4.0-4.5, reflecting its strong acidogenic capacity³⁴.

In summary, lactobacilli play critical roles in food fermentation, flavor development and human health. Their capacity to produce organic acids not only preserves fermented foods but also promotes intestinal health by suppressing pathogenic microorganisms and supporting a balanced gut microbiota.

***Streptococcus thermophilus*:** *Streptococcus thermophilus* is a technologically important lactic acid bacterium widely employed in the dairy industry, particularly in the production of yogurt and a variety of fermented milk products. It functions in a well-established symbiotic relationship with *Lactobacillus delbrueckii* subsp. *bulgaricus*, wherein complementary metabolic activities enhance the efficiency of milk fermentation. *S. thermophilus* synthesizes formic acid, which stimulates the growth of *L. bulgaricus*, while *L. bulgaricus* contributes key flavor compounds such as acetaldehyde and provides proteolytic activity that releases peptides and amino acids required for the growth of *S. thermophilus* in milk³⁵.

The genus *Streptococcus* comprises Gram-positive bacteria that share similar fermentative metabolic pathways but differ substantially in their ecological distribution and physiological characteristics. Over the past two decades, extensive taxonomic revisions have reassigned several species formerly categorized under *Streptococcus* to new genera such as *Enterococcus* and *Lactococcus*. Notably, *S. thermophilus* remains the only dairy-associated species that continues to be classified within the genus *Streptococcus*³⁵. Based on phylogenetic analyses and ecological traits, streptococci are grouped into three major categories: "oral," "pyogenic," and "other streptococci." The oral group includes the *S. mutans*, *S. mitis*, *S. anginosus* and *S. thermophilus* clusters. Within this group, *S. thermophilus* is unique in being exclusively associated with milk and dairy ecosystems. Other Gram-positive cocci frequently present in similar environments include species of *Enterococcus*, *Lactococcus*, *Pediococcus* and *Leuconostoc*³⁶.

Morphologically, *S. thermophilus* cells are spherical to ovoid, approximately 0.7-0.9 µm in diameter and typically occur in pairs or in long chains comprising 10-20 cells. They are Gram-positive, non-motile, non-spore-forming and catalase-negative and they lack cytochromes. This species is homofermentative, producing predominantly L(+)-lactic acid as the main metabolic end product. It is a facultative anaerobe and does not utilize arginine. Optimal growth occurs between 40 and 45°C and the organism exhibits notable thermotolerance, surviving exposure to 60°C for up to 30 min. During milk fermentation, the culture pH commonly decreases to 4.0-4.5, reflecting its strong acid-producing capacity³⁴.

Effects of bifidobacteria and lactobacilli on cheese composition and flavor: The incorporation of probiotic bacteria such as bifidobacteria and lactobacilli into cheesemaking has been investigated for both technological and functional health benefits. In most studies, the inclusion of these microorganisms does not markedly affect the overall chemical composition of cheese³⁷. However, specific probiotic strains may influence certain physicochemical parameters. For example, the addition of *Bifidobacterium lactis* Bb-12 to Cheddar cheese increased moisture content from 38-40%, likely due to accelerated acidification by the strain¹⁶. Although, the higher moisture level supported enhanced bacterial activity, it also resulted in a slight reduction in texture and body quality during sensory evaluation.

Proteolysis in cheese is largely governed by coagulant-derived enzymes and the native milk enzyme plasmin¹⁷. While probiotics typically exert minimal influence on proteolytic patterns, some studies report increased protein degradation

and elevated free amino acid levels following their incorporation, contributing to more complex flavor profiles^{14,16}. The breakdown of caseins yields peptides and amino acids that impart characteristic taste attributes, including acidity, sweetness, bitterness and malty notes. In certain instances, excessive proteolysis can lead to undesirable off-flavors³⁸.

Lipolytic activity-essential for flavor development during cheese ripening-is primarily associated with starter and non-starter lactic acid bacteria. Probiotic strains seldom induce substantial changes in free fatty acid concentrations during maturation, although heterofermentative bifidobacteria and lactobacilli may elevate acetic acid levels as a result of their metabolic pathways^{16,39}. Bifidobacteria typically produce acetic and lactic acids in an approximate 2:3 ratio via the fructose-6-phosphate shunt pathway⁴⁰. Moderate acetic acid production contributes positively to cheese aroma, whereas excessive accumulation may impart sharp, sour, or otherwise undesirable flavor notes⁴¹.

Bifidobacteria also exhibit β -galactosidase activity, facilitating lactose hydrolysis and potentially alleviating lactose maldigestion in sensitive individuals⁴². When incorporated into cheeses such as Crescenza, Canestrato Pugliese and Cheddar-type varieties, these bacteria effectively degrade lactose, resulting in minimal residual galactose content³⁹. Although, the cheese matrix provides a protective environment that enhances probiotic survival during manufacture and ripening^{27,43}, fewer probiotic strains have been successfully adapted for cheese compared with fermented milks and yogurts. Consequently, careful strain selection is essential to ensure microbial viability while preserving desirable sensory attributes.

Recent investigations have also highlighted *Propionibacterium* species as promising probiotic candidates for incorporation into Swiss-type cheeses, offering new opportunities to enhance both the functional and sensory qualities of dairy products¹⁸.

Cheese as a vehicle for probiotic microorganisms: Probiotic foods are designed to deliver live microorganisms that can survive gastrointestinal transit and confer health benefits to the host. For probiotics to be effective, they must remain viable throughout manufacturing, storage and consumption and withstand exposure to harsh digestive conditions such as low pH, gastric acid and bile salts in the small intestine⁴⁴. Among dairy-based probiotic carriers, cheese has been identified as particularly suitable, often outperforming fermented milk products such as yogurt⁴⁵.

The structural and compositional characteristics of cheese create an environment conducive to probiotic stability. Its dense matrix provides a natural buffering effect against gastric acidity, locally increasing the pH surrounding microbial cells and improving their likelihood of surviving digestion⁴⁴. Furthermore, the relatively high fat content of cheese forms a protective barrier that shields probiotic cells from physical and chemical stress during ingestion and gastrointestinal passage^{46,47}.

The enhanced protective capacity of cheese has been demonstrated in several comparative studies. When *Lactobacillus casei* 334e was incorporated into both yogurt and reduced-fat Cheddar cheese, similar initial counts were observed (approximately 7.0 log CFU/g). Under simulated gastric conditions at pH 2.0, viable counts declined to 4.0 log CFU/g after 120 min in Cheddar, whereas in yogurt they decreased to roughly 1.0 log CFU/g. These findings underscore the superior ability of cheese to maintain probiotic viability under acidic stress^{48,49}.

Comparable results have been reported for requeijão, a traditional Portuguese whey cheese. Probiotic strains including *Bifidobacterium animalis*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* and *Lactobacillus brevis* were exposed to simulated gastric conditions (pH 2.5-3.0, 1000 U/mL pepsin and 0.3 g/100 mL bile salts at 37°C). Although, viability decreased, the cheese matrix preserved a substantial proportion of the probiotic population^{50,51}.

Similar observations have been documented for fresco cheese, an Argentinean soft cheese containing mixed cultures of *L. casei*, *Bifidobacterium* spp. and *L. acidophilus*. When exposed to acidic conditions (pH 2 and pH 3), all strains survived for up to three hours. *L. casei* CI exhibited the highest resistance, showing only a two-log reduction at pH 3 and strong survival even at pH 2, whereas *Bifidobacterium bifidum* B4 was the least tolerant⁵².

Milk composition-particularly its lipid fraction-significantly influences cheese texture and the survival of probiotics during ripening. Variations in fat extraction and fatty acid profiles have been documented depending on the analytical methods employed⁵³.

Overall, cheese serves as an advantageous delivery vehicle for probiotic microorganisms due to its buffering capacity, structural density and lipid composition. These characteristics protect bacterial cells during processing, storage and gastrointestinal passage, thereby supporting their viability and enhancing the likelihood that they exert beneficial health effects in the host.

Cheese as a functional food matrix for probiotic delivery:

Cheese is widely recognized as one of the most suitable and efficient food matrices for delivering probiotics to the human gastrointestinal tract. Compared with fermented milk products, cheese provides a more stable and protective environment that enhances probiotic survival during processing, storage and gastrointestinal transit^{54,55}. Its physicochemical characteristics-including relatively high pH, low acidity, compact texture, substantial fat content, strong buffering capacity and limited oxygen permeability-create favorable conditions that support microbial viability and stability throughout the product's shelf life^{14,56}. The incorporation of probiotic cultures into cheeses and other dairy products has also been shown to improve their functional properties⁵⁷⁻⁵⁹.

A wide variety of regional and international cheeses have successfully served as carriers for probiotic microorganisms. These include Ras or Roumy cheese, Mascarpone, Minas fresh cheese, fresh soft cheese, fresh cream cheese with inulin, Festivo, Crescenza, Fresco, cottage cheese, Petit-Suisse and Pategrás cheeses^{60,61}. Additional examples encompass Tallaga, Iranian white cheese, Karish, Cremoso, Gouda, probiotic goat cheese, Canestrato Pugliese, Turkish white cheese, Cheddar, Beyaz, white brined cheese and cheese-based dips^{39,62-67}. A diverse range of probiotic species has been incorporated into these cheese varieties, including *Bifidobacterium animalis* subsp. *lactis*, *B. longum*, *B. bifidum*, *B. infantis*, *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, *L. gasseri* and *Propionibacterium freudenreichii* subsp. *shermanii*¹⁵.

To exert measurable health benefits, probiotic cheeses must deliver adequate numbers of viable cells, generally at least 10 CFU/g at the time of consumption. Therefore, selected strains must withstand the mechanical, thermal and biochemical stresses associated with cheese manufacture, ripening and storage, while preserving stability and viability until ingestion. The performance of each strain depends on both the cheese type and the specific technological parameters applied. Starter lactic acid bacteria typically dominate immediately after production, whereas non-starter lactic acid bacteria gradually increase during maturation⁴⁴. Although maintaining probiotic viability in aged cheeses can be challenging, numerous studies report that viable counts frequently remain above 10 CFU/g at the end of ripening, with only modest reductions during refrigerated storage^{15,68}. These findings highlight the capacity of the cheese matrix to support long-term probiotic survival.

Beyond surviving storage, probiotics must remain viable during passage through the gastrointestinal tract to achieve their intended health effects. Cheese has shown superior performance compared with liquid carriers such as milk in delivering live probiotics to the intestine. Simulated digestion studies have consistently demonstrated this enhanced protective effect. For example, *Bifidobacterium bifidum* and *Lactobacillus casei* incorporated into Fresco (fresh-type), Cheddar (hard-type) and Minas (fresh-type) cheeses exhibited only a 2-3 log reduction in viable counts following exposure to simulated gastric and intestinal conditions⁵².

Ultimately, the success of probiotic cheese depends on the ability to maintain high microbial viability while preserving desirable sensory, chemical and microbiological qualities throughout ripening and storage. Ensuring an optimal balance among these attributes enables production of cheese that reliably delivers health benefits without compromising consumer expectations of taste, texture and overall quality.

Sensory attributes of probiotic versus non-probiotic cheeses:

Maintaining desirable sensory characteristics is essential for the consumer acceptance of probiotic cheeses and dairy products⁶⁹⁻⁷¹. Ideally, probiotic-enriched cheeses should resemble their conventional counterparts in terms of flavor, aroma, texture and appearance. However, the metabolic activity of probiotic strains during fermentation and ripening can modify these sensory attributes, either positively or negatively, depending on the specific strain and manufacturing conditions.

A substantial body of research indicates that the incorporation of probiotic bacteria does not necessarily compromise the sensory quality of cheese. For example, the addition of *Bifidobacterium* species to Cheddar cheese had no significant effects on flavor, texture, or appearance during 24 weeks of ripening⁷². Although, certain *Bifidobacterium* strains increased acetic and lactic acid concentrations, these compositional changes did not translate into detectable sensory differences⁷³. Likewise, cheeses produced with *Lactobacillus paracasei* and *Lactobacillus salivarius* exhibited organoleptic and chemical characteristics comparable to those of control sample⁷⁴. In Minas fresh cheese, *Lactobacillus acidophilus* La-5 did not significantly influence sensory attributes during storage at 5 °C for seven days 40 and when co-cultured with *Streptococcus thermophilus*, it maintained stable flavor and texture for up to 14 days⁷⁵.

Nonetheless, certain probiotic strains may adversely affect sensory acceptance by generating excessive proteolytic or acidic metabolites. Proteolytic enzymes produced by lactic acid bacteria hydrolyze milk proteins into peptides and amino

acids that may impart bitterness or sourness^{12,76,77}. In Cheddar and Ras cheeses, probiotic additions have at times resulted in increased bitterness, vinegary notes, or heightened sourness relative to control cheeses^{14,73}. Specific strains such as *Lactobacillus casei* 279 and *Lactobacillus paracasei* L26 have been associated with higher bitterness scores in Cheddar cheese and lactobacilli more generally have contributed to early sour and bitter flavor development during the initial six months of ripening^{73,78}. Similarly, *Bifidobacterium* strains can occasionally produce off-flavors due to elevated acetic acid formation¹⁶. The inoculation level also influences sensory outcomes; higher concentrations of *L. acidophilus* in Minas fresh cheese led to undesirable alterations in appearance, aroma, flavor and texture⁷⁹.

Conversely, several studies have demonstrated that appropriately selected probiotic cultures can enhance the sensory profile of cheese. For instance, *Lactococcus casei* ssp. *rhamnosus* LBC 80, when combined with *Lactococcus lactis* strains, improved the texture and sensory qualities of reduced-fat Kefalograviera cheese after 3-6 months of ripening⁸⁰. Co-culturing *Lactobacillus delbrueckii* subsp. *bulgaricus* with *Streptococcus thermophilus* has been shown to enhance flavor development in Ras cheese during 90 days of aging¹⁴. Other adjunct probiotics also contribute to improved sensory quality; for example, *Bifidobacterium animalis* ssp. *lactis* Bb-12 produced more desirable flavor notes than *B. longum*⁸¹, while the peptidase activity of *Lactococcus lactis* ssp. *cremoris* enhanced overall flavor balance⁸². Furthermore, the inclusion of *Lactobacillus* adjunct cultures has been associated with improvements in flavor, texture and visual appeal in both Cheddar and Festivo low-fat cheeses^{83,84}.

Overall, the sensory impact of probiotics in cheese is highly strain- and dose-dependent and is shaped by the technological parameters of production and ripening. When carefully selected and appropriately managed, probiotic cultures can maintain, or in some cases enhance, the flavor, texture and overall acceptability of cheese products.

Beneficial and potential effects of probiotic foods

Production of antimicrobial substances: Probiotic microorganisms enhance both food safety and gastrointestinal health through their capacity to produce antimicrobial compounds that inhibit pathogenic and spoilage microorganisms. Beyond their role in acidification, many probiotic strains exert protective effects through the secretion of bacteriocins and other inhibitory metabolites⁸⁵. For example, *Bifidobacterium bifidum* synthesizes bacteriocins with activity against several harmful bacteria, including *Bacillus*, *Listeria*, *Lactobacillus*,

Leuconostoc, *Enterococcus* and *Pediococcus* species⁸⁶. Similarly, numerous *Lactobacillus* and *Lactococcus* species are well recognized for producing bacteriocins—small, ribosomally synthesized peptides with antimicrobial properties that typically target closely related or pathogenic bacteria⁸⁶.

In addition to bacteriocins, many probiotic organisms produce short-chain fatty acids (SCFAs), such as acetic, propionic and butyric acids, during carbohydrate metabolism. These metabolites reduce environmental pH and generate conditions that are unfavorable for the growth of pathogenic microorganisms²⁰. Certain probiotic species can also modulate pathogen virulence. For instance, some strains inhibit the production of Shiga toxin type 2 in enterohemorrhagic *Escherichia coli* O157:H7 via mechanisms associated with organic acid production⁸⁷.

Moreover, several genera of lactic acid bacteria—including *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Lactobacillus* have demonstrated the ability to suppress mycotoxigenic molds, thereby reducing the potential for mycotoxin contamination in fermented and stored food products⁸⁸⁻⁹². Collectively, these antimicrobial mechanisms including bacteriocin synthesis, organic acid production and competitive inhibition play a critical role in improving the microbiological safety and overall functional quality of fermented foods, including cheese.

Immune system modulation: Probiotic microorganisms modulate and enhance the host immune system by activating both innate and adaptive immune pathways. They promote cellular, humoral and nonspecific immune functions through interactions with intestinal epithelial cells and various immune cell populations.

Prevention and treatment of gastroenteritis: Probiotic microorganisms play a significant role in reducing both the incidence and severity of gastroenteritis. Numerous studies have reported that probiotics can shorten the duration of illness and alleviate symptom intensity across various forms of infectious diarrhea⁹³. Their protective effects are mediated through several mechanisms, including the stimulation of host immune defenses, competitive exclusion of pathogens at intestinal epithelial binding sites and the secretion of antiviral and antimicrobial substances that suppress pathogen replication²¹. Beyond gastroenteritis, probiotics have demonstrated therapeutic potential in preventing and managing additional gastrointestinal disorders, such as *Helicobacter pylori* infection, irritable bowel syndrome and inflammatory conditions including Crohn's disease^{3,94,95}. Evidence further suggests that multi-strain probiotic

formulations may offer enhanced therapeutic efficacy compared with single-strain preparations in mitigating symptoms of inflammatory bowel disease⁹⁶.

Reduction of serum cholesterol: Probiotic microorganisms-particularly species of *Lactobacillus* and *Bifidobacterium*-have been shown to reduce serum cholesterol levels by modulating intestinal lipid metabolism. Proposed mechanisms include direct assimilation of cholesterol during microbial growth, adsorption of cholesterol to bacterial cell surfaces, enzymatic deconjugation of bile salts and the bioconversion of cholesterol into bile acids that are subsequently excreted from the body^{19,97}. *In vivo* evidence supports these cholesterol-lowering effects, with strains such as *Lactobacillus plantarum*, *Bifidobacterium longum* and *Enterococcus faecium* reported to improve lipid profiles by reducing total and low-density lipoprotein (LDL) cholesterol while increasing high-density lipoprotein (HDL) cholesterol. These findings indicate that select probiotic strains may serve as effective adjuncts or alternatives to conventional therapies for managing hypercholesterolemia^{98,99}.

Anti-carcinogenic effects: Probiotic microorganisms have been associated with potential anti-carcinogenic properties, as demonstrated *in vivo* studies involving animals and humans and *in vitro* investigations using cancer cell lines¹⁰⁰. Their protective effects are thought to arise from several mechanisms, including modulation of immune responses, attenuation of inflammation and enhancement of immune-mediated suppression of carcinogenic processes. Additionally, certain probiotic strains may bind or metabolize carcinogenic compounds within the gastrointestinal tract, thereby reducing their absorption and mutagenic potential. However, anti-carcinogenic efficacy is highly strain-specific and variations in study design, dosage and duration pose challenges for comparative interpretation. While current findings are encouraging, comprehensive clinical trials remain necessary to validate these effects and to clarify the long-term influence of specific probiotic strains on cancer prevention and therapy¹⁰¹.

Other beneficial effects: Regular consumption of probiotic microorganisms confers a range of additional health benefits beyond gastrointestinal and immunological modulation. Probiotics can beneficially alter the intestinal microbiota of patients with conditions such as leukemia, enhance intestinal motility and reduce the absorption of ammonia and biogenic amines, thereby decreasing toxic load and improving overall metabolic homeostasis. They further support lactose

digestion through the production of β -galactosidase, which is particularly advantageous for individuals with lactose intolerance^{87,102}. In addition, probiotics have been reported to mitigate certain food allergies, synthesize essential vitamins-especially those within the B-complex group-and enhance calcium absorption and bioavailability. Their proteolytic activity enables partial predigestion of dietary proteins, thereby improving the nutritional value and digestibility of foods. Probiotics may also contribute to infection prevention by inhibiting pathogenic colonization and promoting urinary tract health through competitive exclusion and the production of antimicrobial substances.

Side effects and safety of probiotics: Probiotics, particularly species belonging to the genera *Bifidobacterium* and *Lactobacillus*, are widely recognized as safe (GRAS) for human consumption. Adverse reactions in healthy individuals are uncommon and are generally mild when they do occur¹⁰³. Numerous studies have demonstrated their safety during pregnancy and early infancy, indicating that probiotic supplementation does not pose significant health risks in these populations¹⁰⁴. Nonetheless, probiotics may occasionally contain trace allergens originating from production substrates, such as proteins derived from cow's milk or hen's eggs, which may trigger allergic responses in sensitized individuals¹⁰⁵. Although rare, probiotic-associated infections have been documented, primarily among immunocompromised adults, while invasive infections in children or infants remain exceedingly uncommon¹⁰⁶⁻¹⁰⁸.

Health benefits of probiotic cheese: Probiotic cheeses are receiving increasing scientific and commercial interest due to their capacity to promote human health through diverse biological mechanisms. Numerous studies have demonstrated that the incorporation of probiotic microorganisms into cheese can support the prevention and management of gastrointestinal infections, improve lactose digestion, reduce serum cholesterol, enhance immune responses and modulate inflammatory and carcinogenic pathways^{15,27,109}. Additional benefits include antimicrobial activity, anti-diarrheal effects and suppression of *Helicobacter pylori* infection. Importantly, these health-promoting effects are highly strain-specific, underscoring the importance of selecting appropriate probiotic species and maintaining their viability throughout cheese manufacture and storage^{110,111}. To ensure consistent functional performance, probiotic cultures must retain biological activity without compromising the sensory and physicochemical characteristics of the cheese. Moreover, *in vivo* evaluation is necessary to confirm probiotic survival

during maturation, storage and gastrointestinal transit. When utilizing multi-strain formulations, potential synergistic or antagonistic interactions must also be assessed to optimize efficacy¹¹².

Experimental evidence from both animal and human studies supports the physiological benefits of consuming probiotic cheeses. For example, *Enterococcus faecium* Fargo 688 exhibited superior viability in Cheddar cheese relative to yogurt, illustrating the protective role of the cheese matrix. Similarly, a fresh Argentinean cheese enriched with *Lactobacillus acidophilus* A9, *Bifidobacterium bifidum* A12 and *Lactobacillus paracasei* A13 enhanced immune responses in mice, increasing phagocytic activity in the small intestine and raising IgA cell counts in the large intestine after only five days of consumption. These findings indicate that probiotic bacteria can function as antigenic stimuli in the gut, activating both cellular and humoral immune pathways^{113,114}.

The benefits of probiotic cheese extend to oral health as well. In one study, Edam cheese fortified with *Lactobacillus rhamnosus* LC705 and *L. rhamnosus* GG (LGG) was shown to reduce *Streptococcus mutans*, a principal etiological agent of dental caries. After three weeks of regular consumption, the probiotic group exhibited a 21% reduction in *S. mutans* counts, compared to an 8% reduction in the control group, accompanied by increased salivary *Lactobacillus* levels—suggesting improved oral microbial balance and reduced caries risk²². Positive outcomes have also been documented in elderly individuals with oral candidosis. In a clinical trial involving 92 participants, consumption of cheese containing *L. rhamnosus* GG, *L. rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp. *shermanii* JS for 8–16 weeks resulted in a 75% reduction in salivary yeast counts, whereas the control group experienced a 34% increase. Probiotic intake additionally improved saliva quality by raising mucin and immunoglobulin levels, indicating enhanced oral defense mechanisms and potential protection against xerostomia (dry mouth)²².

CONCLUSION

Cheese continues to serve as an effective carrier for probiotic delivery owing to its favorable physicochemical attributes, including high buffering capacity, compact matrix structure and substantial lipid content, all of which support microbial survival during processing, storage and gastrointestinal transit. The incorporation of probiotic strains such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus thermophilus* can enhance both the functional properties of cheese and consumer health by contributing to antimicrobial

activity, cholesterol reduction, improved lactose metabolism and modulation of immune responses. When appropriately selected, these strains can be introduced without causing significant alterations in sensory quality. The viability and functional performance of probiotics in cheese are influenced by multiple factors, including strain-specific characteristics, manufacturing parameters and storage conditions. Overall, probiotic-enriched cheese represents a convergence of traditional dairy technology and contemporary functional food development, offering a stable, palatable and efficient vehicle for delivering live beneficial microorganisms. Such products support gut health, strengthen immune function and contribute to general well-being, while aligning with increasing consumer interest in nutritious, health-promoting foods.

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